UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MAR 0 4 2016

MEMORANDUM

SUBJECT: Environmental risk assessment for the FIFRA Section 3 registration of the TGAI *Bacillus amyloliquefaciens* strain PTA-4838 (EPA File Symbol 70664-T) and two end-use products, Varnimo® WSP (EPA File Symbol 70664-A) and Varnimo® ST (EPA File Symbol 70664-L); Submission No. 958320, Decision No. 495952, DP Barcode No. 424149

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Ecological Risk Assessment

A. Introduction

LidoChem, Inc. has submitted an application to register a new active ingredient, *Bacillus amyloliquefaciens* strain PTA-4838 (hereafter, *B. amyloliquefaciens* PTA-4838), for use as a suppressant against phytopathogenic fungi and nematodes and also for use as plant growth promoter on bushes, crops, herbs, spices, ornamentals, shrubs, trees (forest and shade), turf, vegetables and vines. *B. amyloliquefaciens* PTA-4838 is the active ingredient (a.i.) in two end-use products (EPs), Varnimo® WSP and Varnimo® ST. Varnimo® WSP is a water suspendable powder containing 0.29% a.i. at a minimum of 2×10^9 cfu/g; Varnimo® ST is a powder

containing 73.4% a.i. at a minimum of 5 x 10^{11} cfu/g and is a biological seed treatment that provides early protection against selected parasitic nematodes.

Members of the genus *Bacillus* are endospore forming bacteria. Endospores are formed under adverse growth conditions and help the *Bacillus* species persist in diverse environments (Logan and de Vos, 2009). *B. amyloliquefaciens* is a common bacterium present in soil, water and plant materials and is known to produce many secondary metabolites. EPA registered other strains of *B. amyloliquefaciens* (strains D747 and FZB24) which were reclassified from *B. subtilis* var. *amyloliquefaciens* (Priest et al., 1987; USEPA, 2010; USEPA, 2011).

B. amyloliquefaciens PTA-4838 is a versatile bacterium with multiple and unique modes of action. This strain produces many enzymes with special functions (e.g., phytase, chitinase, lipase and protease) and several secondary metabolites including antifungal lipoproteins (MRID No. 49474102). It is a new a.i., and thus requires a review to assess the potential risks to nontarget organisms that may result from its use. This memorandum contains the ecological risk assessment for the registration of the new a.i. and its proposed uses.

B. Summary of Nontarget Effects

Table 1 provides the status of the Tier 1 Nontarget Organism Data Requirements for the active ingredient *B. amyloliquefaciens* PTA-4838 for ecological risk assessment. Nine original guideline studies with the TGAI and two sets of scientific rationale were submitted to meet data requirements for non-target organisms per 40 CFR Part 158.2150. Relevant information from the submitted and cited studies is included in the discussion of risk below (see C. Ecological Exposure and Risk Characterization) and Data Evaluation Records (DERs) for the submitted guideline studies are attached for this memorandum.

Table 1. Summary of data submitted to comply with Nontarget Organism Data Requirements per 40 CFR Part 158.1250 in support of the Section 3 registration of *B. amyloliquefaciens* PTA-4838.

Data Requirement	**OPPTS Guideline**	**Results Summary and Classification**	**MRID No.**
Avian oral toxicity/pathogenicity	885.4050	Based on the two avian oral studies submitted, *B. amyloliquefaciens* strain PTA-4838 is not toxic or pathogenic to Northern bobwhite (*Colinus virginianus*) dosed at 9.46 x 10^9 cfu/ml/bird/day. However, the birds were not tested at a maximum hazard dose. **Classification: Supplemental**	49474109 49474110

Data Requirement	OPPTS Guideline	Results Summary and Classification	MRID No.
Avian Oral	885.4050	Scientific rationale provided is sufficient to support the requested waiver. **Classification: Acceptable**	49474111
Wild mammals	885.4150	Wild mammal testing was not conducted, but studies required by 40 CFR Part 158.2140 are adequate for assessment of hazards to wild mammals. *B. amyloliquefaciens* PTA-4838 was found not toxic or pathogenic to laboratory rats when tested at 3.5 $x10^8$ cfu/rat by oral gavage (MRID No. 49474105) and at 2.7 $x10^8$ cfu/rat by intravenous injection (MRID No. 49474107). **Classification: Acceptable**	N/A
Freshwater fish toxicity/pathogenicity	885.4200	A 30-day static renewal test with *B. amyloliquefaciens* PTA-4838 had a negative effect on growth of juvenile Rainbow trout (*Oncorhynchus mykiss*) dosed at 1 x 10^6 cfu/mL and on survival at 1 x 10^7 cfu/mL. However, for 100% mortality observed at 1 x 10^7 cfu/mL within 15 days of testing, it is not possible to determine whether the effect was seen because of inherent toxicity/pathogenicity of the a.i. or due to experimental design (e.g., too young fish were used in the testing). **Classification: Supplemental**	49474112
Freshwater invertebrate toxicity/pathogenicity	885.4240	A freshwater invertebrate study with *B. amyloliquefaciens* PTA-4838 showed potential adverse effects on reproduction of *Daphnia magna* at exposure to 1 x 10^5 cfu/mL of test medium in a 21-day static renewal system. Maximum hazard testing was not conducted; however,	49474113

Data Requirement	OPPTS Guideline	Results Summary and Classification	MRID No.
		enumerations indicated exposure at 10^5 cfu/mL. **Classification: Supplemental**	
Nontarget Insect Testing	885.4340	A laboratory bioassay with adult ladybird beetles (*Hippodamia convergens*) at the maximum hazard dose showed relatively high mortality (>20%) of *B. amyloliquefaciens* PTA-4838 to the test organism in **all** groups in 13 days of testing. The results are inconclusive. **Classification: Unacceptable**	49474117
Nontarget Insect Testing	885.4340	A laboratory bioassay with adult parasitic wasp (*Aphidius coleman*) at the maximum hazard dose showed toxic or pathogenic effects of *B. amyloliquefaciens* PTA-4838 to the test organism in **all** groups within 3 days of testing. The diet consisted of 10% honey water with or without the test substance. The study is not of sufficient duration to be useful, and results are inconclusive. **Classification: Unacceptable**	49474118
Nontarget Insect Testing	885.4340	A laboratory bioassay with green lacewing larvae (*Chrysoperla rufilabris*) showed no toxic /pathogenic effect to the test organism at 1.1 x 10^8 cfu/cm^2 within 19 days of testing. The test substance and inactive test substance groups ate > 30% fewer corn earworm eggs *(Helicoverpa zea*) on Day 2 than the un-dosed control group, but these differences were not reported to be significantly different. **Classification: Acceptable**	49474116
Honey bee testing	885.4380	A 13-day laboratory bioassay with "young" honey bees (*Apis mellifera*) showed a relatively	49474119

Data Requirement	OPPTS Guideline	Results Summary and Classification	MRID No.
		high mortality in all test groups. It was determined that *B. amyloliquefaciens* PTA-4838 was not toxic to honey bees at a rate of 10,000X MFAR or 1 x 10^{11} cfu/mL/sq ft when administered orally. **Classification: Supplemental** given the duration (13 days) of the toxicity/pathogenicity testing, the stated dose rate, the rate of mortality (> 20%) in the control group and the test substance group, and high variability shown in the amount of food provided for consumption.	
Estuarine/marine fish and invertebrate testing	885.4280	A 30-day static renewal test with grass shrimp (*Paleomonetes vulgaris)* showed no chronic toxic/ pathogenic effect of *B. amyloliquefaciens* PTA-4838 when tested at concentrations of 10^5 cfu/g diet and 10^6 cfu/g diet. The EC_{50} for Varnimo® Technical was determined to be > 10^6 cfu/g, based on mortality observations. While there was no significant difference in the weight or length of the shrimp among the test groups, contamination of the controls makes the results less useful for a risk assessment. **Classification: Supplemental**	49474114
Estuarine/marine fish and invertebrate testing	885.4280	A 30-day laboratory test with inland silverside (*Menidia beryllina*) dosed at 10^6 cfu/mL and 10^7 cfu/mL showed potential chronic toxic and/or pathogenic effect at 10^7 cfu/mL in a 30-day static renewal system. Based on mortality data (40% mortality at 10^7 cfu/mL), the LC_{50} for Varnimo® Technical is > 1x 10^6 cfu/mL. However, the submitted	49474115

Data Requirement	OPPTS Guideline	Results Summary and Classification	MRID No.
		study is insufficient to determine whether other sublethal effects may result from exposure to the active ingredient. **Classification: Supplemental**	
Nontarget plant Testing	885.4300	A data waiver from nontarget plant testing requirement for *B. amyloliquefaciens* PTA-4838 was submitted. Scientific rationale based on published literature is not sufficient to support no adverse effects to all plants. **Classification: Unacceptable** Additional information is needed to support claims of non-persistence and lack of phytotoxicity.	49474309

C. Ecological Exposure and Risk Characterization

The EP Varnimo® WSP is a water suspendable powder that is intended for use as a suppressant against certain fungal pathogens and/or parasitic nematodes on bushes, crops, herbs, spices, ornamentals, shrubs, trees, turf, vegetables and vines, and/or as plant growth promoter. This EP can be applied alone or in combination and/or rotation with chemical fungicides as a tool for integrated disease management in agricultural crops, ornamentals, nursery plants and turfgrass. Varnimo® WSP may be used at the following rates: For foliar applications, 3-32 ounces Varnimo® WSP mixed in a minimum of 25 gallons of water can be applied per acre (1.9×10^7 cfu/mL). For drench/drip irrigation applications, 3-32 ounces Varnimo® WSP in a minimum of 25 gallons of water can be applied. The EP Varnimo® ST is a biological seed treatment that provides early season protection against selected plant parasitic nematodes. For seed treatment, it can be applied up to 0.09 ounces per 100 lbs seed, resulting in ca.1×10^6 cfu/g seed.

The EP Varnimo® WSP is recommended to be applied in pre-emergence (e.g., 2-7 days prior to planting) as sprays, hy chemigation (e.g., sprinkler, drip, drench, flood, in-furrows and borders), or by direct applications (e.g., as pre-planting, at-planting and post-planting amendments). There is not much drift from sprinkler irrigation, and in most chemigation applications, watering in is required, which would reduce runoff. All of the chemigation applications are soil directed and soil-directed applications with Varnimo® WSP are generally expected to have less potential for nontarget exposure compared to other applications (e.g., aerial sprays). Additionally, for pre-emergence uses, the label directs the user to irrigate immediately after treatment and to keep soil moist until planting.

For post-emergence applications (e.g., foliar and soil-directed applications), Varnimo® WSP is expected to have more potential for nontarget exposure, since nontarget organisms and/or their food items may be directly sprayed with the product. However, it is noted that irrigation immediately after application is required on the label to move the product into the root zone, so exposure on foliar surfaces within the treated area will be short lived. This does not eliminate exposure to soil-dwelling organisms. Additionally, some drift is expected from foliar sprays, and the label is not explicit about whether aerial applications may be made. Aerial spray applications would be expected to result in greater amounts of the product drifting off-site compared to ground spray applications.

The EP Varnimo® ST is intended for seed treatment which is likely to result in low exposure of nontarget organisms to the product. Birds may be exposed by direct consumption of seeds, and soil-dwelling organisms may also be exposed to some degree. It is noted, however, that *B. amyloliquefaciens* occurs naturally in soil, water and plant materials, so soil-, water-, and plant-borne non-target organisms in those areas currently have some exposure to the existing *B. amyloliquefaciens* strains.

Birds and Mammals

The guideline study submitted for the avian oral toxicity/pathogenicity testing requirement showed no adverse effects in birds (Northern bobwhite, *Colinus virginianus*) when tested at 9.46 x 10^9 cfu/ml/quail/day. *B. amyloliquefaciens* strain PTA-4838 is not known to be pathogenic to birds.

The spray concentration resulting from the highest application rate (32 oz) mixed in the minimum amount of water (25 gallons) based on the Varnimo WSP label is 1.9 x 10^7 cfu/mL (the product contains at least 2 x 10^9 cfu/g). This concentration is below the level at which birds were tested and shown to have no adverse effects. Exposure by direct consumption of treated seed is also expected to be below this level, since a bird would need to consume >1000g of seed per day to achieve the same level of exposure as the birds in the study describe above. Based on expected field application rate and lack of adverse effects observed in birds exposed to *B. amyloliquefaciens* strain PTA-4838 at the level tested, risk to nontarget birds resulting from the proposed registration is not expected. Since birds were not tested at the maximum hazard dose, any future increases in application rate of *B. amyloliquefaciens* PTA-4838 would need to be evaluated for bird risks.

An acute oral toxicity/pathogenicity study with laboratory rats indicated no adverse effects of *B. amyloliquefaciens* strain PTA-4838 when administered at a dose of 3.5 x 10^8 cfu/rat (MRID No. 49474105). An acute intravenous injection study (MRID No. 49474107) was also submitted, which showed that *B. amyloliquefaciens* strain PTA-4838 was non-toxic and not infective to rats at a dose of 2.7 x 10^8 cfu/animal (USEPA, 2016).

Some wild mammals may be exposed to *B. amyloliquefaciens* strain PTA-4838 in treated areas; however, adverse effects were not observed in the mammalian studies. Therefore, risk to wild mammals is expected to be low from the proposed registration of the new a.i.

Freshwater Fish and Invertebrates

A guideline study submitted for the non-target freshwater fish testing requirement showed an effect of *B. amyloliquefaciens* PTA-4838 on *Oncorhynchus mykiss* (rainbow trout) when tested at 1 x 10^6 cfu/mL and 1 x 10^7 cfu/mL. The a.i. was found to have an adverse effect on survival (e.g., 100% mortality within 15 days of testing) of the test organisms at 10^7 cfu/mL, and an adverse effect on growth (e.g., weight and length) of the test organisms at 10^6 cfu/mL. 100% mortality seen at 10^7 cfu/mL, but not at 10^6 cfu/mL, appears to be dose-dependent, hence the a.i. shows a toxic effect under the conditions tested. However, for other observations (e.g., growth rate, abnormal behavior/appearance, and weight), it is not possible to determine whether the effects were seen because of inherent toxicity/pathogenicity of the a.i. or due to experimental design. The study report indicates that the water in the 10^7 cfu/mL test substance group was heavily cloudy with no visibility on many days. The necropsy showed that many fish in this group showed discoloration, indicating stress. Many of the fish also died suddenly (e.g., 10 died in one replicate on day 5 due to loss of air supply, 19 died on day 15 for reasons not explained). No follow-up work was performed to determine if the mortality was the result of pathogenic effects. It is possible that the effects observed were due to toxicity and/or pathogenicity, but may also have been the result of the physical conditions produced by the high concentration of the test substance.

A guideline study for the non-target freshwater invertebrate testing indicated no significant effect on mobility (indicator of mortality), but showed a potential toxic/pathogenic effect of *B. amyloliquefaciens* strain PTA-4838 on reproduction of *Daphnia magna* when tested at 1 x 10^5 cfu/mL. The maximum hazard dose (testing at 1 x 10^6 cfu a.i. /mL) was not used in the study, and old and new test media were contaminated in some cases. However, the contamination was not described as the test substance, and it is possible that other microbes entered the non-sterile test environment. It is unclear whether there may be effects from the contamination that was detected; however, conclusions regarding lack of mortality are considered reliable.

The EPA Standard Wetland, which is a wetland of 1 acre in size and 15 cm in depth, can be used to determine a worst-case scenario estimate for EEC in water. The EEC for water is calculated to be 3 x 10^3 cfu/ml ([32 ozs (product/acre) X (28.35 g/oz) X (2 X 10^9 cfu/g product) X (1 acre/40,468,564 cm^2)] ÷ 15 cm). Using the EPA Standard Pond, which is a pond of 1 acre in size and 6 ft (182.88 cm) deep, the EEC would be 2.5 x10^2 cfu/mL. To gain a more realistic EEC based on the potential for spray drift, the Tier I aerial agricultural application scenario in the AgDRIFT spray drift model can be applied to these numbers. Under that scenario assuming fine to medium droplet size, a maximum of 12.5% of the amount applied is expected to reach aquatic areas at the water's edge. Therefore, the EEC for the wetland and pond can be refined to 3.8 x

10^2 cfu/mL and 3.1 x 10^1 cfu/mL, respectively, and this exposure is expected to be even lower with ground spray applications. Since the potential for exposure falls three orders of magnitude or more below the 10^5 cfu/mL from the *Daphnia* study described above, and the 10^6 and 10^7 cfu/mL in the rainbow trout study above, adverse effects to aquatic animals in freshwater systems are not expected.

B. amyloliquefaciens PTA-4838 produces a biosurfactant (surfactin), chitinase and other secondary metabolites (MRID No. 49474102). Surfactants are reported to be toxic to fish and invertebrates (Abel, 1974; Abel and Skidmore, 1975) and chitinase can be toxic to invertebrates containing chitin as a structural component. Some adverse sublethal effects were observed in the studies described above, and there is some uncertainty as the level of exposure at which such effects might occur. These metabolites likely were included in the test material used in the studies above, and they may have contributed to the effects observed. However, as described above, exposure to the a.i. in aquatic environments is expected to ≥ 1000X below the levels tested. Therefore, any effect of these metabolites on aquatic organisms is expected to be minimal. Since the maximum hazard dose was not tested in the *Daphnia* study, any future increases in the application rate would need to be reevaluated for potential effects on these aquatic organisms.

Marine/Estuarine Fish and Invertebrates

The applicant submitted two guideline studies to meet the requirement for nontarget marine/estuarine fish and invertebrate testing. This testing is conditionally required if significant exposure of nontarget marine/estuarine fish and invertebrates to *B. amyloliquefaciens* strain PTA-4838 is expected; however, EPA did not specifically require these studies.

A 30-day static renewal test was conducted with grass shrimp (*Paleomonetes vulgaris*) to determine the chronic toxicity/pathogenicity of *B. amyloliquefaciens* PTA-4938 at concentrations of 10^5 cfu/g diet or 10^6 cfu/g diet. The active ingredient was shown to have no significant difference in mortality and in tbe weight/length of the shrimp among different test groups under the conditions tested. The EC50 was determined to be > 10^6 cfu/g, based on mortality observations. However, because of contamination in the un-dosed control and the sterile filtrate control, it is not possible to discern whether the test material had an effect on weight or length.

A 30-day static renewal test was conducted with inland silverside (*Menidia beryllina*) to determine the chronic toxicity/pathogenicity of *B. amyloliquefaciens* PTA-4938 at concentrations of 10^6 cfu/mL or 10^7 cfu/mL in the test water (these groups were also exposed via diet at 10^5 cfu/g and 10^6 cfu/g diet, respectively). A mortality rate of 40% was shown in the test organisms when exposed at 10^7 cfu/mL, but no significant mortality (96.7%) was seen when exposed at 10^6 cfu/mL. Based on mortality observations, the LC50 is determined to be > 10^6 cfu/mL. *B. amyloliquefaciens* PTA-4938 showed no statistically significant difference in the weight or length of the silverside among different test groups when tested at10^6 cfu/mL or 10^7 cfu/mL. The test organisms in **all** groups lost 40-60% in weight and 9-13% in length. It is not clear

whether these are sublethal effects that resulted from exposure; however, since the fish were shown to have shrunk in length (an unusual result for studies with these animals). These observations suggest a husbandry issue or poor measurement technique at the testing laboratory. Contamination with potential test substance in the un-dosed control and sterile filtrate groups, introduces uncertainty in the observations of sublethal effects, so these results are not considered reliable

Similar to a case with the nontarget organisms in freshwater environments, *B. amyloliquefaciens* PTA-4838 is not expected to reach marine/estuarine environments in significant quantities, and exposure is expected to be >1,000X below the levels at which effects were tested in the above studies. Even though there is uncertainty related to the sublethal observations in these studies, adverse effects are expected to be very minimal, given the low level of exposure in these environments.

Nontarget Insects and Honey Bees

A guideline study submitted for the requirement for nontarget insect testing showed an effect to ladybird beetles (*Hippodamia convergens*) upon a 13-day dietary exposure to corn earworm (*Helicoverpa zea*) eggs sprayed with *B. amyloliquefaciens* PTA-4838 at the maximum hazard dose. Adult ladybird beetles were used and their age was not specified. The use of the adult insects of unknown age can affect the study results. Relatively high mortality rates (> 20%) were shown in **all** groups, including the control group, in 14 days, but toxicity of the test substance could not be clearly demonstrated. The results of the study are inconclusive and the study is unacceptable.

A guideline study with green lacewing (*Chrysoperla rufilabris*) larvae did not show a toxic/pathogenic effect to the test organism in a 19-day dietary exposure at the maximum hazard dose of *B. amyloliquefaciens* PTA-4838. There appears to be much less (30%, Table 3 of the MRID No. 49474116) amount of eggs consumed in the inactive and active test substance groups than in the un-dosed control group, but these differences were not reported to be significantly different.

A 3-day bioassay study with parasitic wasp (*Aphidius colemani*) showed toxic effects of *B. amyloliquefaciens* PTA-4838 to the test organism when tested at the maximum hazard dose. The study ended in 3 days due to high mortality observed in the control group (77%) and >90% in both the test substance and inactive test substance groups. The results are inconclusive as no differences in mortality were found among different test groups.

A guideline study with honey bees (*Apis mellifera*) was conducted for 13 days. *B. amyloliquefaciens* PTA-4838 was determined to be not toxic to honey bees when administered orally at a stated concentration of 10,000X the maximum field application (MFAR) or 1×10^{11} cfu/ml/sq ft in 50% sugar solution. It is noted that the Agency calculation indicates the dose rate is much lower than the stated 10,000X MFAR, but was still well above the maximum hazard

dose. The mortality observed in the control group and the test substance group on day 13 were 23% and 25%, respectively. It was stated in the study (MRID No. 47474119) that "young" bees were tested, but specific information regarding age was not provided. When young bees are used, the bees need be newly emerged in order to obtain the results useful for a risk assessment. Exposure to honey bees may occur with applications to foliage; however, based on the study, potential hazard to bees is not expected.

Exposure to nontarget insects is expected in the treated areas. Nontarget insects may be directly sprayed within treated areas, or they may contact treated surfaces. Exposure through contact with treated foliage is expected to be short-lived within areas targeted for treatment, since the label requires immediate irrigation of treated plants to move the product off of foliar surfaces and into the root zone. Therefore, insect exposure in the treated area is primarily expected to occur through direct contact with spray during application or through contact with soil in treated areas. Within soil, exposure is expected to be somewhat spatially limited, since *B. amyloliquefaciens* PTA-4838 is expected to inhabit plant roots once applied. *B. amyloliquefaciens* PTA-4838 may persist with formation of endospores; however, it is noted that *B. amyloliquefaciens* is a common inhabitant of soil and nontarget insects likely are already exposed at some level to this bacterium.

Foliar sprays are likely to result in exposure to nearby areas through drift of the applied product. In these areas, exposure will be lower, since a fraction of the sprayed amount is expected to drift. However, unlike treated areas that will be immediately irrigated after treatment, *B. amyloliquefaciens* PTA-4838 is expected to remain on foliar surfaces for a longer period of time after application. Therefore, exposure at some level cannot be avoided in these areas.

While exposure is expected to be somewhat limited in treated areas, the potential for exposure to nontarget insects cannot be ruled out. Data available for other nontarget insects are incomplete, since two of the submitted studies were inconclusive. One study indicates that applications of *B. amyloliquefaciens* PTA-4838 may be relatively safe for certain nontarget insects. However, as described above, *B. amyloliquefaciens* is known to produce secondary metabolites and/or enzymes that may adversely affect insects, and several recent reports in the literature indicate the potential for use of this bacterium or its biosurfactants in insect control (Gadhave and Gange, 2016; Khedher et al., 2015; Li et al., 2015; Yun et al. 2013). On the other hand, *Bacillus amyloliquefaciens* has been isolated from the gut of certain insects (see USEPA, 2010). Concerns for nontarget insects had previously been raised for a currently registered strain of *B. amyloliquefaciens*, but EPA determined that the weight of the evidence indicated that adverse effects were not expected (USEPA, 2010). However, given the varying information regarding the potential for adverse effects on nontarget insects, including more recent reports, the lack of acceptable data on this specific strain, and the potential for exposure particularly associated with foliar sprays, adverse effects to nontarget insects cannot be ruled out with high certainty. Removal of foliar sprays from the proposed label would significantly reduce exposure to nontarget insects both within the treated areas and in adjacent areas. This application method may be better supported in the future with additional acceptable testing on insects.

Nontarget Plants

A guideline study for Nontarget Plant Testing, Tier 1 was not submitted, but a data waiver was requested for the new a.i. *B. amyloliquefaciens* grows in association with plants (Alvarez et al., 2011) and is a plant growth-promoting bacterium (Chen et al., 2007), but is not a known plant pathogen. Few adverse effects of *B. amyloliquefaciens* on plants have been reported by the applicant from the published literature on existing *B. amyloliquefaciens* strains on greenhouse, growth chamber or field studies (MRID No. 49474309). Scientific rationale submitted for a waiver of the requirement for Nontarget Plant Testing should be strengthened as some references were either too general or outdated to be useful. The rationale provided was insufficient to conclude that the proposed uses of *B. amyloliquefaciens* PTA-4838 are not expected to result in increased exposure to, or adverse effects in nontarget plants of economic importance.

Bacillus amyloliquefaciens produces secondary metabolites that may have adverse effects on certain nontarget organisms (e.g., fish, aquatic invertebrates, insects – see discussion above). Its reported biology (e.g., an endospore former) and the wide range of use sites and application methods may argue against the applicant's claim of no adverse effects and nonpersistence of the active ingredient in all environments. Additionally, Talboys et al. (2014) reported reductions in inorganic phosphorus (Pi) uptake by *Triticum aestivium* seeds treated with *B. amyloliquefaciens* FZB42, resulting from the action of auxin produced by this strain in low Pi environments. These effects were not observed in high Pi environments, where the effect was observed to be beneficial. While this paper may not signal potential for adverse effects in all conditions, the authors conclude the importance of understanding bacteria-plant interactions when deciding among pesticide alternatives. More information like this would better inform users of biopesticide products and also future pesticide risk assessments.

Exposure to nontarget plants is expected with the proposed registration of *B. amyloliquefaciens* PTA-4838, particularly with foliar sprays that may cause drift or to nontarget plants on the field that may contact *B. amyloliquefaciens* in the soil. *B. amyloliquefaciens* is not a known plant pathogen and is a common soil bacterium that has a close association with many plant species. Adverse effects to nontarget plants also have not been documented. Therefore, adverse effects are not anticipated from the proposed registration of the products containing this a.i.

D. Environmental Persistence

Bacillus amyloliquefaciens is a common bacterium found in soil, water and plant materials. Like other *Bacillus* species, it forms endospores under adverse growth conditions. Endospores can survive extreme environmental conditions (e.g., heat, desiccation and chemicals) that would normally kill the bacterium, which makes the bacterium persistent in diverse environments. It is also known to produce special enzymes and secondary metabolites, including antibiotics and antifungal lipoproteins (Alvarez et al., 2011; Chen et al., 2007).

Bacillus amyloliquefaciens PTA-4838 is a nutritionally and biochemically versatile bacterium with multiple and unique modes of action. This strain produces many enzymes with special functions (e.g., phytase, chitinase, lipase and protease) and several secondary metabolites such as fengycin, iturin, and surfactin (MRID No. 49474102). The EPs likely contain both cells with endospores and vegetative cells. Therefore, the active ingredient is expected to persist in the environment for a prolonged period once introduced. Whether it persists at levels that are likely to have pesticidal benefits or adverse effects is uncertain.

E. Federally Listed Threatened and Endangered Species

Based on available information, EPA has determined that no adverse effects are anticipated for birds, mammals, fish, aquatic invertebrates, and plants exposed to *B. amyloliquefaciens* PTA-4838 as a result of the proposed labeled applications. Therefore, a "No Effect" determination is made for direct and indirect effects to listed species of these taxa and their designated critical habitats resulting from the proposed registration of *B. amyloliquefaciens* PTA-4838. Concerns were raised for nontarget insects for the proposed registration of *B. amyloliquefaciens* PTA-4838 as labeled, due to uncertainties related to lack of acceptable data and reports of potential effects in the literature. Therefore, direct effects to listed insects cannot be precluded, nor can indirect effects to listed obligate insectivores whose food supply may be affected. However, adverse effects are not expected to nontarget insects if foliar applications are removed from the proposed Varnimo® WSP label, as this is expected to significantly reduce exposure to nontarget insects. In such case, EPA could make a "No Effect" determination for direct effects to listed insects and for indirect effects to listed obligate insectivores.

References

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